Evaluation of a rapid Cryptosporidium/Giardia immunochromatographic test for diagnosis of giardiasis in dogs.

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Introduction

Giardia is a cosmopolitan enteric protozoan with a very wide host range, including domestic and wild animal species as well as human beings. As the extent of morbidity caused by Giardia and its potential for zoonotic transmission have become better understood, an increasing importance has been placed on the methods of this parasite detection in animals’ reservoirs, including dogs. Detection of Giardia infections in dogs can be achieved by the zinc sulphate concentration technique that is commonly regarded as the best diagnostic method. However, morphological detection of Giardia cysts in faecal samples by this procedure is technically difficult and time consuming, and requires high degree of client compliance. Indeed, the sensitivity of the zinc sulphate concentration technique is approximately 70% on a single step due to the intermittent excretion of cysts over time, and at least three faecal samples have to be obtained over a 3-5 day period to enhance the rapid detection of Giardia. One of these, the RIDA® Quick Cryptosporidium/Giardia-Combi test has been developed by R-Biopharm AG (Darmstadt, Germany).

SUMMARY:

The aim of this study was to evaluate the performance of the RIDA® Quick Cryptosporidium/Giardia-Combi test (R-Biopharm AG) as a rapid method for the routine diagnosis of giardiasis in dogs. Compared to an ELISA coproantigen test (Prospect® Giardia Microplate Assay), the immunochromatographic test had a sensitivity of 83%, a specificity of 79%, a positive predictive value of 33%, and a negative predictive value of 97%. The k value was 0.5. Although the immunochromatographic test is simpler to perform, it cannot replace the Giardia ELISA coproantigen test for rapid diagnosis of giardiasis in dogs, since its sensitivity and specificity appear to be not comparable to those of the ELISA kit. No Cryptosporidium infection was detected.

Key-words: Giardia, Immunochromatographic test, ELISA, dog, faeces.
This test relies on the detection of cell wall proteins of *Giardia* cysts and trophozoites using monoclonal antibodies. The test is performed on fresh or thawed stool and requires a 3-min settling time and 5-min incubation time at room temperature. Infection with *Giardia* is indicated by the appearance of a band and the result can be visually read by non-specialized personnel. The aim of the present study was to evaluate the RIDA® Quick Cryptosporidium/Giardia-Combi test as a rapid initial method for diagnosis of *Giardia*-osis in dogs. The results were compared to those obtained using a commercial ELISA coproantigen test.

**Materials & methods**

**SAMPLES**

Fresh stool samples were obtained from 164 dogs with suspected intestinal parasitosis presented at our Veterinary Medical Teaching Hospital (Department of Veterinary Clinic, University of Pisa, Italy) or at different private veterinary practises in the Pisa area. All samples were examined for the presence of *Giardia* infection using the RIDA® Quick Cryptosporidium/Giardia-Combi test and an ELISA coproantigen kit. Fresh stool specimens were stored at +5 °C after collection and examined within 2-3 days. If fresh specimens could not be tested within 2-3 days, they were frozen at -20 °C. Frozen stool specimens were thawed at +5 °C temperature before use, and were examined within 1 hour after complete thawing. In all the cases, the RIDA® Quick Cryptosporidium/Giardia-Combi assay was performed and evaluated before ELISA tests were processed.

**IMMUNOCHROMATOGRAPHIC TEST :**

The RIDA® Quick Cryptosporidium/Giardia Combi test (R-Biopharm AG, Darmstadt, Germany) was used. This test uses monoclonal antibodies directed against specific membrane proteins of *Cryptosporidium* and against specific cell wall proteins of cysts and trophozoites of *Giardia*. The method is a one-step lateral flow immunochromatographic assay containing specific antibodies labelled to coloured latex particles (red for *Giardia* and blue for *Cryptosporidium*) to generate a visual signal. Latex labelled specific antibodies are also applied at different levels on the membrane proteins of cysts and trophozoites of *Giardia*. The method is performed properly [6].

**ANALYSIS OF RESULTS :**

Proportions of positive samples and corresponding 95 % confidence intervals were calculated for each of the two tests.
Sensibility, specificity, positive predictive value, and negative predictive value of RIDA® Quick Cryptosporidium/Giardia-Combi test were determined. The sensitivity is the probability that the assay will be positive when the infection is present. The specificity is the probability that the assay will be negative when the infection is absent. They were calculated using the following formulas: sensitivity (%) = TP/(TP+FN) x 100 and specificity (%) = TN/(TN+FP) x 100 (TP: true positive, FN: false negative, TN: true negative and FP: false positive values). The positive predictive value of a diagnostic test is the proportion of total positive test results that are true positives. The negative predictive value of a diagnostic test is the proportion of total negative results that are true negatives. These were calculated using the following formulas: positive predictive value (%) = TP/(TP+FP) x 100 and negative predictive value (%) = TN/(TN+FN) x 100 (TP: true positive, FN: false negative, TN: true negative and FP: false positive values). The kappa (k) statistic value that expresses the agreement beyond chance between tests was also calculated. By convention, the significance of kappa values was the following: 0.0-0.2: slight agreement; 0.2-0.4: fair; 0.4-0.6: moderate; 0.6-0.8: substantial and 0.8-1.0: almost perfect agreement between tests. The diagnostic gains from positive and negative test results were 22 % and 86 %, respectively.

Cryptosporidium infections were not detected in the present study. In approximately 6 % of the cases, a colour other than blue appeared in the positive line to Cryptosporidium, with faint to very faint intensity. Based on information given by the manufacturer, these were due to an excess of faecal material and thus were considered as Cryptosporidium negative results.

Discussion

To our knowledge, this is the first study that uses the RIDA® Quick Cryptosporidium/Giardia-Combi test to detect Giardia infection in dogs. Our study indicates that the test has not a comparable sensitivity to ELISA (83 % vs. 98-100 %), and also that its specificity (79 %) is rather low. Therefore, these findings clearly suggest that the use of RIDA® Quick Cryptosporidium/Giardia Combi assay would lead to a high proportion of dogs being misdiagnosed (as false positives or false negatives). By contrast, the manufacturer claims that the RIDA® Quick Cryptosporidium/Giardia Combi assay is highly sensitive (100 %) and specific (95.2 %) in human faeces when compared to microscopy. The reason of this discrepancy with dogs is unknown and could be due to some factors present in dog faeces. Otherwise, since this kit is geared for rapid diagnosis in humans, the reduced level of sensibility and specificity may also result from infections of dogs with isolates of Giardia that were antigenically undetectable by the assay. It is known that the ELISA test is able to detect a Giardia antigen (GSA 65) that is a glycoprotein common to all the isolates of Giardia spp., whereas only cell wall proteins of Giardia cysts and trophozoites are detectable by the immunochromatographic kit. Anyway, the RIDA® Quick Cryptosporidium/Giardia-Combi test can not replace the Giardia ELISA coproantigen kit for the diagnostic purpose in dogs. Further, other faecal antigen tests such as SNAP Giardia (IDEXX Laboratories), already exist for the in-clinic rapid detection of Giardia in dogs and they have been shown to be highly sensitive and specific [7].
Sometimes, the RIDA® Quick Cryptosporidium / Giardia-Combi test was difficult to evaluate. In some cases, faint to very faint *Giardia* positive red bands were seen. In other cases, a colour other than blue appeared in the Cryptosporidium positive line. Searching in manufacturer’s instructions, no indication was found for such eventualities. Therefore, when both these situations occurred, it was needed to contact the manufacturer directly and ask for information in order to give a final interpretation of the results.

The RIDA® Quick Cryptosporidium Giardia-Combi test allows simultaneous detection of both *Cryptosporidium* and *Giardia* infections on the same strip by different bands. However, in the present survey, *Cryptosporidium* infection was not detected in any of the samples. This is in agreement with previous results [3, 10]. In Italy, the presence of cryptosporidiosis has been established in humans [2], cattle [5], and birds [11]. Reports from dogs are very limited in this country. Canestri Rotti [5] detected *Cryptosporidium* oocysts in 1 out of 15 puppies. TRALDI [12] reported a case of infection in an adult dog. Our negative results suggest that *Cryptosporidium* infections of dogs are probably not common in Italy, or the prevalence of canine cryptosporidiosis is very low in the area examined. The possible occurrence of zoonotic transmission between humans and companion animals has been hypothesised [4]. Since this possibility appears to be mostly associated with farm livestock [4], it would probably be interesting to examine dogs from rural environments and to compare data with our study carried out in dogs living in urban settlements.

With the RIDA® Quick Cryptosporidium/Giardia-Combi test, a number of samples can be processed quickly and with minimum effort. Compared to microscopy, direct immunofluorescent test, ELISA, or PCR, the technological expertise necessary to perform the assay is minimal. The test might be used for the rapid diagnosis of giardiasis in dogs by personnel not adequately trained in laboratory procedures, and in less well-equipped laboratories too. Because it could be used by general veterinarians, results could be read immediately, and thus therapeutic measures could rapidly be carried out, this assay might be of practical use in veterinary practises. Therefore, we conclude that research into developing a more sensitive and specific RIDA® Quick Cryptosporidium / Giardia-Combi test should be pursued to allow the use in veterinary medicine, because there are many potential advantages of such a tool with respect to other diagnostic methods.

References